Fish HSP70 ELISA kit

Cat. No. KITG-085 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit uses a one-step double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Samples, standards, and the HRP-labeled detection antibody are sequentially added to microwells pre-coated with an antibody against fish heat shock protein 70 (HSP-70), followed by incubation and thorough washing. TMB substrate is then added. Under peroxidase catalysis, TMB develops a blue color and changes to yellow after acidification. The color intensity is positively correlated with the amount of HSP-70 in the sample. Absorbance is measured at 450 nm, and the sample concentration is calculated.
Species	Fish
Conjugation/Label	HRP
Description	Assay Type: One-step double-antibody sandwich ELISA. Species: Fish. Analyte: Heat shock protein 70 (HSP-70). Detection Wavelength: 450 nm. Standard Concentrations (S0-S5): 0, 100, 200, 400, 800, and 1600 pg/mL. Detection Range: 10-1600 pg/mL. Sensitivity: The minimum detectable concentration is less than 10 pg/mL. Accuracy: The correlation coefficient R between the linear regression of the standards and the expected concentrations is greater than or equal to 0.9900. Specificity: No cross-reactivity with other soluble structural analogs. Repeatability: Both intra-assay and inter-assay coefficients of variation are less than 15%. Format: 96-well or 48-well. For Research Use Only. Not for use in clinical or human experiments.
Form	Kit

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Applications	Quantitative determination of fish heat shock protein 70 (HSP-70) in serum, plasma, cell culture supernatants, and tissue homogenates.
Notes	Sample Collection, Processing, and Storage 1. Serum: Use pyrogen-free and endotoxin-free tubes. Avoid any cell stimulation during handling. After blood collection, centrifuge at 3,000 rpm for 10 min and rapidly and carefully separate the serum from the erythrocytes. 2. Plasma: Use EDTA, citrate, or heparin as the anticoagulant. Centrifuge at 3,000 rpm for 30 min and collect the supernatant. 3. Cell Culture Supernatant: Centrifuge at 3,000 rpm for 10 min to remove particles and aggregates. 4. Tissue Homogenate: Add an appropriate amount of physiological saline to the tissue and homogenize. Centrifuge at 3,000 rpm for 10 min and collect the supernatant. 5. Storage: If samples will not be tested promptly after collection, aliquot them in single-use portions and store at -20°C. Avoid repeated freeze-thaw cycles. Thaw at room temperature and ensure that the samples are completely and uniformly thawed. Required Materials Not Supplied 1. Microplate reader capable of measurement at 450 nm. 2. High-precision pipettes and tips: 0.5-10 µL, 2-20 µL, 20-200 µL, and 200-1000 µL. 3. 37°C incubator. Operating Precautions 1. Store the kit at 2-8°C and equilibrate it at room temperature for 20 min before use. Crystallization may occur in the concentrated wash buffer; this is normal. Warm the buffer in a water bath until the crystals are completely dissolved before use. 2. Return unused strips immediately to the resealable bag, seal the bag, and store under cool, dry conditions. 3. The S0 standard with a concentration of 0 may be used as the negative control or blank. Samples are diluted fivefold during the procedure; multiply the final calculated result by 5 to obtain the actual sample concentration. 4. Perform all incubations strictly according to the specified times, liquid volumes, and sequence. 5. Mix all liquid components thoroughly before use. Plate Washing 1. Manual Washing: Remove all liquid from the wells, fill each well with wash buffer, allow the plate to stand for 1 min, remove the liquid, and blot the plate dry on absorbent paper. Repeat for a total of 5 washes. 2. Automatic Plate Washer: Dispense 350 µL wash buffer into each well,

	soak for 1 min, and wash the plate 5 times. Assay Procedure 1. After the foil pouch has equilibrated at room temperature for 20 min, remove the required strips. Seal the remaining strips in the resealable bag and return them to 4°C. 2. Set up standard wells and sample wells. Add 50 µL of the appropriate standard concentration to each standard well. 3. Add 10 µL of the sample to each sample well, followed by 40 µL of Sample Diluent. Do not add liquid to the blank well. 4. Except for the blank well, add 100 µL of HRP-labeled Detection Antibody to each standard well and sample well. Seal the reaction wells with a plate sealer and incubate at 37°C in a water bath or incubator for 60 min. 5. Discard the liquid and blot the plate dry on absorbent paper. Fill each well with wash buffer, allow the plate to stand for 1 min, discard the wash buffer, and blot the plate dry. Repeat for a total of 5 washes. An automatic plate washer may also be used. 6. Add 50 µL of Substrate A and 50 µL of Substrate B to each well. Incubate at 37°C in the dark for 15 min. 7. Add 50 µL of Stop Solution to each well. Measure the OD value of each well at 450 nm within 15 min. Result Calculation In a spreadsheet, plot the standard concentration on the x-axis and the corresponding OD value on the y-axis. Generate the linear regression curve for the standards and calculate each sample concentration from the regression equation. Because samples are diluted fivefold during the assay, multiply the calculated value by 5 to obtain the actual sample concentration. The procedure must be followed strictly.
Storage	Kit Components and Storage 96-Well Format / 48-Well Format: Pre-coated Microplate: 12 wells × 8 strips / 12 wells × 4 strips. Standards: 0.3 mL × 6 tubes / 0.3 mL × 6 tubes. Sample Diluent: 6 mL / 3 mL. Detection Antibody-HRP: 10 mL / 5 mL. 20× Wash Buffer: 25 mL / 15 mL. Substrate A: 6 mL / 3 mL. Substrate B: 6 mL / 3 mL. Stop Solution: 6 mL / 3 mL. Plate Sealers: 2 sheets / 2 sheets. Instruction Manual: 1 copy / 1 copy. Resealable Bag: 1 / 1. Storage: 2-8°C, protected from light and moisture. Shelf Life: 6 months.

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Reconstitution	20× Wash Buffer Preparation: Dilute the 20× Wash Buffer 1:20 with distilled water by mixing 1 part 20× Wash Buffer with 19 parts distilled water.
Reaction conditions	Equilibrate the kit at room temperature for 20 min. Add 50 µL standard to each standard well, or add 10 µL sample followed by 40 µL Sample Diluent to each sample well. Add 100 µL Detection Antibody-HRP to all standard and sample wells, excluding the blank well, and incubate at 37°C for 60 min. Wash 5 times, with a 1-min soak during each wash. Add 50 µL each of Substrate A and Substrate B and incubate at 37°C in the dark for 15 min. Add 50 µL Stop Solution and read absorbance at 450 nm within 15 min.

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